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Toxic and Gonadotropic Effects of Cadmium and Boron Relative to Standards for These Substances in Drinking Water

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Experimental research was conducted on the study of the relationship of the general toxic and gonadotoxic effects of cadmium and boron under conditions of subacute and chronic oral intoxication on white random-bred rats, by use of biochemical, physiological, cytological, and pathomorphological methods. It is shown that the gonadotoxic effect of cadmium is manifested on the same level (3 mg/kg of body weight) as the general toxic effect. The gonadotoxic effect of boron is dominant and is manifested at a lower level (6 mg/kg of body weight) than the general toxic effect (20 mg/kg). On the basis of the results of chronic experiments, 0.001 mg/l. is recommended as the hygienic standard for cadmium in water and 0.5 mg/l. for boron.

In recent years, evidence has accumulated in the literature on the gonadotropic effect of cadmium and boron (*1*–*7*). In earlier studies conducted in the U.S.S.R., the hygienic standards for the content of these substances in water were established without consideration of their effect on gonad function. For this reason, we have set for ourselves the task of making the threshold and nonactive levels of these compounds in water more precise while taking into consideration their effect on the functional state of the organism and the state and function of the gonads.

In addition to a general toxic effect, a gonadotropic effect was determined for a cadmium dose of 1–4 mg/kg of weight, depending on the species of animal, in recalculating to ions of the metal and for a dose of 6 mg/kg in the case of boron under conditions of subacute oral cadmium chloride and boric acid intoxication for an average period ranging from 21 to 35 days. The general toxic effect of cadmium was shown by the reduction of peroxidase and blood cholinesterase activity and in the reduction of the sulfhydryl group count in the blood serum of experimental animals. The general toxic effect of boron was shown by the reduced activity of the aldolase of blood serum. The gonadotropic effect of both metals was observed in subacute experiments based on changes in the function and histostructure of the gonads. Thus the histological analysis of the gonads in the case of cadmium intoxication made it possible to explain the intensification of desquamation and the disorganization of the spermatogenic epithelium, hypertrophy and the proliferation of Sertoli cells, thickening of basal membranes, swelling of arteriolar walls and reduction of the arteriolar lumen, expansion and congestion of the capillary network, formation of a transudate, and the appearance of multinuclear giant cells in the lumen of the spematic cords as a result of the disturbance of endomytosis for the effect level of the metal. Total

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damage to the seminiferous tubules was observed in a number of cases. All of the above changes were accompanied by proliferation of Leydig cells and by the appearance of lymphoid cells in individual fields. Significant thickening of the tunica was observed in a number of cases. The functional state of spermatozoa changed under the effect of large doses of cadmium and boron: there was diminished movement, reduced mobility time, a decrease in the acidic and osmotic resistance of the spermatozoa, and a reduction in the number of spermatozoa.

Studies were conducted by using the method of Clermont and Leblond (*8*) to determine the sensitivity of the various cells of the spermatogenic epithelium to the effect of cadmium in doses of 3, 9, and 27 mg/kg of body weight. The stage of each section of a tubule was determined and the number of tubules at a given stage of development of the seminal epithelium from L to XIV was counted. Round tubules were taken into account. In all, we counted 1000 seminal tubule sections. The percentage survival after the action of cadmium was taken as the primary criterion for the sensitivity of the various cells of the seminal epithelium.

Type A spermatogonia which exist in all of the stages from I to XIV were considered for all of the stages. Type B spermatogonia and spermatocytes which are present at various stages of meiosis prophase exist and divide at given stages of the seminal epithelium cycle. For this reason, in order to count these cells, we chose 20 sample batches at those stages at which the given type of cells would be present in the norm. The number of morphologically normal cells was counted at each section of the tubules.

Data obtained on the survival percentage of various types of spermatogonia (Table 1) show that the effect of cadmium in 3 and 27 mg/kg doses does not result in significant changes in the quantitative composition of all types of spermatogonia. Analysis of data dealing with the effect on primary spermatocytes, which were at the stages of proleptonema, leptonema, zygonema, and pachynema, did not show changes in the number of morphologically normal cells of this type in the seminal epithelium of rats (Table 2).

Thus the sensitivity of the method used and the results of the cytogenetic study made it possible to conclude that there was no effect of cadmium on the early developmental stages of the spermatogenic epithelium, i.e., spermatogonia and spermatocytes.

Since the results of subacute experiments proved that boron and cadmium exhibit a gonadotropic effect, we conducted a chronic 6-month experiment with the daily introduction of cadmium chloride in doses of 0.00005, 0.0005, and 0.005 mg/kg of animal body weight to random-bred white male rats with an average weight of 300 g. This corresponds to a cadmium chloride content in water at the level of 0.001, 0.01, and 0.1 mg/l. (calculated for ions of the metal).

Table 1.

Dose, mg/kg of animal body weight	Number of spermatogonia								
	Type A			Intermediate type			Type B		
	All cells	Average per section	T/C[a]	All cells	Average per section	T/C[a]	All cells	Average per section	T/C[a]
Control	880	3.79		844	10.68		2661	32.06	
3	1085	4.34	1.14	1089	11.7	1.09	2459	31.93	1
27	593	3.31	0.87	627	10.62	0.99	2141	36.91	11

[a]Test/control

Table 2.

Dose, mg/kg animal body weight	Number of spermatocytes											
	Proleptonemae			Leptonemae			Zygonemae			Pachynemae		
	All	Average	T/C[a]	All	Average	T/C[a]	All	Average	T/C[a]	All	Average	T/C[a]
Control	4905	50.6		942	55.5		1710	58.96		14304	63.3	
3	5079	52.9	1.04	5686	61.1	1.1	3379	63.75	1.08	15574	62.7	0.99
27	2970	51.2	1.01	2033	69.8	1.07	1448	62.73	1.06	10038	66.0	1.04

[a]Test/control.

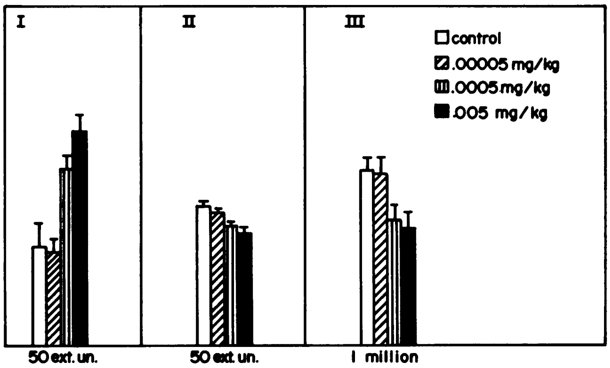

FIGURE 1. General toxic effect of cadmium: (I) alkaline phosphatase; (II) SH group in blood serum; (III) erythrocyte count.

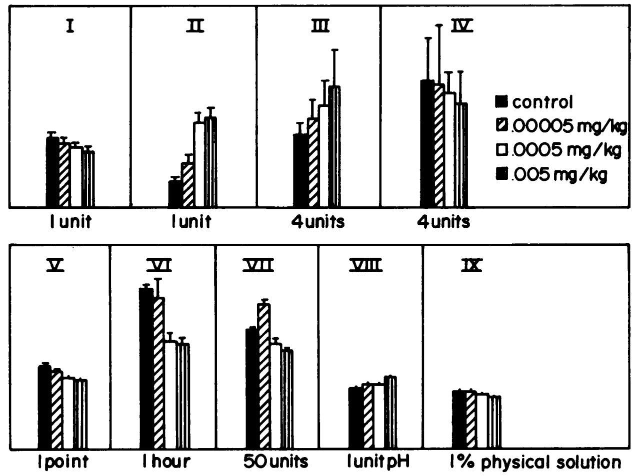

FIGURE 2. Gonadotropic effect of cadmium: (I) spermatogenesis index; (II) number of tubules with 12th meiosis stage; (III) number of tubules with cast-off epithelium; (IV) number of spermatogonia; (V) motility of spermatozoa; (VI) mobility time; (VII) spermatozoa count; (VIII) acid resistance; (IX) osmotic resistance of spermatozoa.

Cadmium at a dose of 0.005 mg/kg of body weight was shown to cause a reduction in animal weight (420 ± 5.1 for the control group and 378 ± 4.8 for the test group, p (<0.01), a reduction in the sulfhydryl group content in blood serum, an increase in alkaline phosphatase activity, erythropenia (Fig. 1), and a reduction in the β-lipoprotein content of blood serum. These changes started occurring during month 2-3 of exposure. During the last month of the experiment, we recorded a small increase in the amount of carbon dioxide in the blood, an increase in nitrogen in the urea (14 mg−% for the control group and 17 mg−% for the test group) and a reduction in the inorganic phosphorus content (6.65 mg−% for the control group and 5.06 mg−% for the test group). A tendency was noted towards increased glycolysis and an increase in the amount of cadmium in the blood (70mg−) for the control group and 95mg−% for the test group). The overall protein and albumins in the blood did not change. An increase was observed in the weight coefficients of the internal organs, particularly in the case of the liver and the adrenals (2.0 ± 0.002 for the control group and 2.27 ± 0.021 for the test group with $p < 0.01$). The function and structural indices of the gonads changed (Table 3 and Fig. 2). The index of spermatogenesis fell (3.57 ± 0.046 in the case of the control group and 2.71 ± 0.015 for the test group with $p < 0.01$). An increase was observed in the number of tubules with cast-off epithelium (13.4 ± 1.0 for the control group and 22.86 ± 3.4 for the test group with $p < 0.05$). An increase was also observed in the number of tubules with a 12th stage meiosis (1.43 ± 0.29 for the control group and 4.29 ± 0.47 for the test group with $p < 0.01$). The

Table 3. Function of the gonads during chronic action of cadmium introduced orally.

Dose, mg/kg of animal weight	Motility of spermatozoids	Mobility time of spermatozoids, hr.	Number of spermatozoids	Acid resistance of spermatozoids, pH units	Osmotic resistance of spermatozoids, % of solution	Weight coefficients of the gonads[a]	
						P	L
Control	4 ± 0.01	7.65 ± 0.25	285 ± 0.25	2.93 ± 0.008	2.7 ± 0.1	37.5 ± 0.05	37.8 ± 0.04
0.00005	3.7 ± 0.08	7.17 ± 0.95	345 ± 13	3.05 ± 0.161	2.7 ± 0.1	38.8 ± 0.08[b]	37.2 ± 0.008[c]
0.0005	3.4 ± 0.09[d]	5.09 ± 0.5[c]	249 ± 14[d]	3.03 ± 0.04[b]	2.6 ± 0.002[d]	41.8 ± 0.002[d]	41.4 ± 0.01[d]
0.005	3.28 ± 0.08[d]	5.04 ± 0.26[d]	235 ± 9.1[c]	3.39 ± 0.069[b]	2.5 ± 0.002[d]	35.7 ± 0.078[c]	34.6 ± 0.04[b]

[a](Weight of gonads $R_g \times 10^4$)/weight of animals R_{zh}.
[b]$p < 0.01$.
[c]$p < 0.05$.
[d]$p < 0.001$.

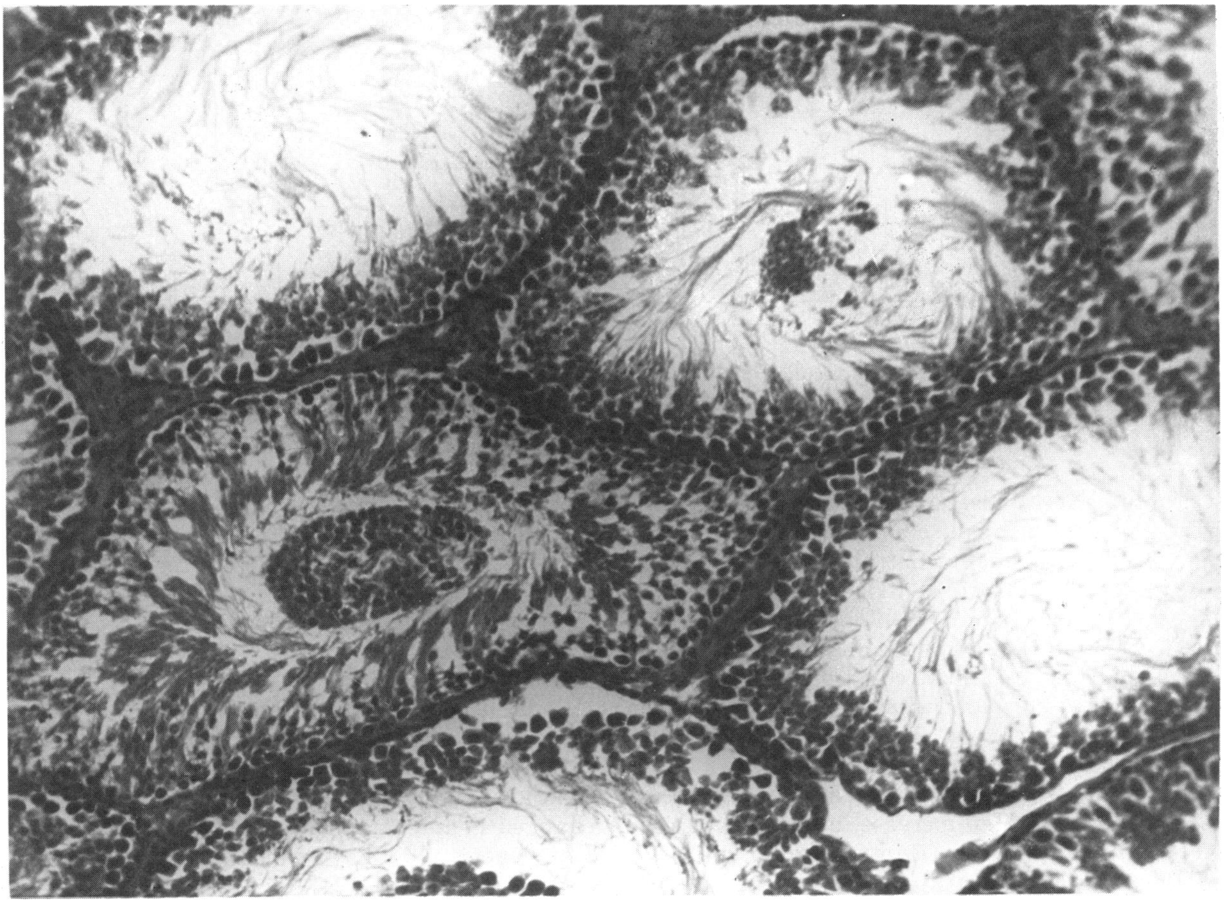

FIGURE 3. Tubules with cast-off epithelium and peeling of the spermatogenic epithelium from the basal membranes. Cadmium dose, 0.005 mg/kg of body weight; hematoxylin-eosin stain; 200×.

following changes were observed in the histostructure of the gonads in studying the index of spermatogenesis: desquamation of the spermatogenic epithelium and its detachment from the basal membrane (Fig. 3). Changes in the conditional reflex activity of experimental animals were caused by cadmium in 0.005 and 0.00005 mg/kg doses (Table 4). Cadmium at levels of 0.0005 mg/kg of animal body weight caused changes in a number of the indices for the functional state of the experimental animals and the gonads for practically the same periods of intoxication, but to a lesser extent. Such indices as the concentration of the ions of potassium, sodium, and chlorine in the blood, overall proteins and albumins, creatinine, cholesterol, and overall blood bilirubin did not produce any significant changes in comparison to the control group. Even in the case of a cadmium dose of 0.05 mg/kg, no changes were detected in the peroxidase, transaminase and lactate dehydrogenase activity of the blood or in gonad size. Changes in the weight factors of the gonads bore an equidirectional character: an increase was observed for a small dose and a reduction in the case of a large dose.

From these data it follows that the presence of the gonadotropic effect is characteristic in the case of cadmium intoxication both for large and small doses. This gonadotropic effect is accompanied by a general toxic effect, even at the threshold dose level. Thus a 0.0005 mg/kg dose can be considered the threshold, while a 0.00005 mg/kg dose (which corresponds to 0.001 mg/l.) can be considered a no-effect dose which can be recommended as the hygienic norm for water reservoirs.

Table 4. State of higher nervous activity of rats during chronic intoxication with cadmium chloride introduced orally.

	Process characteristics							
	Formation							
$CaCl_2$ concn, mg/l.	Manifestation	Consolidation	Latent period, sec	Value of conditioned reflex, ma	Value of unconditioned reflex, ma	% of conditioned reflex present	Decrease in conditioned reflex, ma	Restoration of reflex action
Control	10.75 ± 1.1	26.9	1.27 ± 0.2 1.0 ± 0.1	30.9 ± 4.42 31.3 ± 0.9	41.0 ± 1.2 39.8 ± 0.28	94 ± 1.88	13 ± 0.7	4.4 ± 0.6
0.001	10.0 ± 1.0	25.6	1.07 ± 0.17 0.82 ± 0.09	33.7 ± 1.52 31.7	42.8 37.7 ± 1.8	96.05 ± 1.25	14 ± 0.6	4.0 ± 0.64
0.01	12.4 ± 1.11[a]	27.3	1.76 ± 0.16[b] 1.18 ± 0.01	26.4 ± 1.75 28.3	38.6 32.2 ± 1.85	87.34 ± 2.78[b]	10.6 ± 1.096[b]	1 ± 0.6[b]
0.1	16.37 ± 1.55[a]	27.0	1.85 ± 0.16[b] 1.25 ± 1.86[b]	26.7 ± 1.86[b] 26.7	34.6 31.7 ± 1.9[b]	87.09 ± 2.29[b]	9.0 ± 1.291[b]	1.6 ± 1.3[a]

[a] $p < 0.01$.
[b] $p < 0.05$.

In discussing the results we should keep in mind that Lenskaya's data (*9*) from the study of the action of cadmium on the formal elements of the blood and on the glycogen-forming function of rabbits fed the metal in doses of 0.0005 and 0.005 mg/kg of body weight served as the basis for the existing hygienic standard. Since Lenskaya had not obtained significant changes, cadmium was not studied as an element which was dangerous to the health of the population, and its standard was set at the 0.01 mg/l. level for the reservoir water. In our study, this value turned out to be active, exhibiting both general toxic and gonadotropic effects. This is in agreement with data in the literature which point out that during the itai-itai epidemic in Japan, the water in the Dzhintsu River basin showed a cadmium content of 0.009 mg/l. The no-effect dose for cadmium established by us for water reservoirs is in agreement with that which was recommended by the working group of the European Regional Bureau of VOZ, 9 January 1973. The recommendations of this working group stated that a 0.005 mg/l. cadmium concentration must be considered as the maximally inactive concentration for the human organism.

In an analogous toxicological experiment with boron, random-bred white male rats with the same weight as above received boric acid in aqueous solution from graduated feeders in doses of 0.015, 0.05, and 0.3 mg/kg (converted to ions of boron). The control group received tap water.

The state of the experimental animals was followed by determining the weight of the animals, the peroxidase, cholinesterase, and aldolase activity in the blood; the β-lipoproteins and sulfhydryl group content in blood serum, and aspartate- and alaninamine-transferase activity. After the 6-month experiment was completed, research was conducted on the study of the gonadotropic effect of boron with respect to the functional weight coefficients and size of the gonads. DNA content was determined for the gonads, spleen, and the adrenals. The conditioned reflex activity of the experimental animals was studied under chronic experiment conditions (Table 5).

Table 5. State of the higher nervous activity of rats during chronic intoxication with boric acid.

	Number of combinations						
Concentration of boron in water, mg/l.	Manifestation of conditioned reflex	Strengthening of conditioned reflex	Latent period, sec	Value of conditioned reflex, ma	Value of unconditioned reflex, ma	Decrease in conditioned reflex, ma	Restoration
Control	5.85 ± 0.64	21.42 ± 0.84	1.30 ± 0.04	36.84 ± 0.77	37.14 ± 1.25	29.6 ± 1.53	5.3 ± 0.7
0.3	5.57 ± 0.65	22.42 ± 0.84	1.21 ± 0.08	36.69 ± 1.58	34.19 ± 1.05	28.6 ± 1.64	6.1 ± 0.9
1.0	5.28 ± 0.97	23.42 ± 1.07	1.28 ± 0.064	34.36 ± 1.02	34.26 ± 1.27	33.4 ± 1.65	6.4 ± 0.4
6.0	4.85 ± 0.73	27.6 ± 1.44[a]	1.48 ± 0.06[b]	35.18 ± 1.84	34.19 ± 1.02	37.6 ± 1.97[b]	5.9 ± 1.1

[a] $p < 0.01$.
[b] $p < 0.05$.

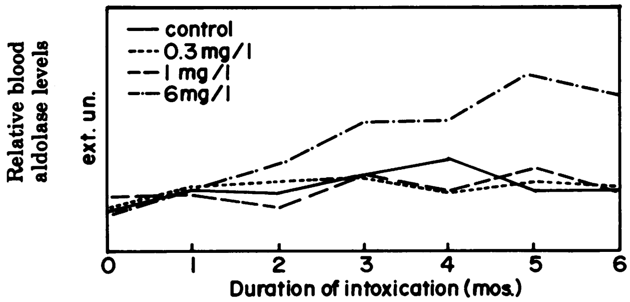

FIGURE 4. Toxic effect of boron as a function of time as shown by aldolase activity of blood serum in white rats.

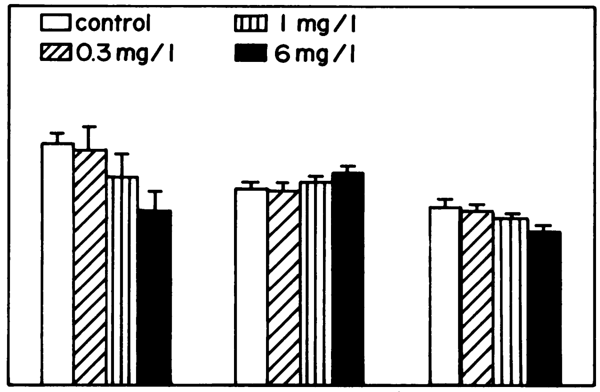

FIGURE 5. Gonadotoxic effect of boron.

Analysis of the experimental results, indicates that boron in 0.3 mg/kg doses produces increased blood aldolase activity (241 ± 4.95 for the control group and 307.9 ± 4.98 for the test group, $p < 0.01$). This increase was already sharply expressed during the second month of intoxication (Fig. 4). A reduction was also noted in the weight factors of the gonads and the mobility time of the spermatozoids. In addition, boron caused a decline in the mobility of the spermatozoids, a reduction of their number and acid and osmotic resistance (Table 6, Fig. 5). The DNA content of raw gonad tissue was reduced (2.54 ± 0.12 mg/g for the control group and 1.46 ± 0.24 mg/g for the test group).

A 0.05 mg/kg boron dose exhibited a weakly expressed gonadotropic effect which was manifested in the reduction of spermatozoid mobility time and in a decreased spermatozoid count.

Nevertheless, a number of the indices of the functional state of the gonads for these animals did not differ from those of the control group: weight factors, nature of the motion of the spermatozoids, and osmotic resistance.

Animals which had been subjected to the two highest boron doses showed a reduced lactic acid content of the liver (*in situ*), 18 and 29% respectively. These animals showed a tendency towards a drop in liver glycogen. In conjunction with this, in studying glycolysis *in vitro;* the glycolysis processes totally normalized in the homogenates of the liver during the introduction of such factors as NAD, ATP, and Mg^{2+}. Consequently it can be assumed that boron inhibits glycolysis in an intact liver not by blocking the glycolytic enzymes, but by blocking the cofactors of this process. The oxidation phosphorylation of the liver mitochondria was practically unchanged for the animals of all of the three groups. There was also no change in the mitochondrial protein content.

The results obtained under conditions of chronic enteral intoxication proved that boron has a gonadotropic effect and that the 0.015 mg/kg dose should be considered inactive.

Since three local drinking water sources were observed with a boron content on the level of 0.015,

Table 6. Changes in the function of the gonads during chronic action of boron orally introduced.

Concentration of boron, mg/l.	Weight coefficients of gonads[a]	Mobility time of spermatozoids, min	Motility of spermatozoids	Acid resistance of spermatozoids, pH units	Osmotic resistance of spermatozoids, % of solution	Number of spermatozoids
Control	40.14 ± 0.55	380 ± 11.04	4.0	3.07	2.75 ± 0.11	196 ± 2.8
0.3	39.64 ± 1.22	370 ± 18	3.9	3.06 ± 0.075	2.72 ± 0.099	196 ± 2.16
1.0	38.72 ± 0.58	328.7 ± 21.3[b]	4.0	3.22 ± 0.047[b]	2.6 ± 0.09	174 ± 1.49[c]
6.0	35.57 ± 1.16[c]	275 ± 16.4[d]	3.1	3.36 ± 0.075[e]	2.4 ± 0.05[e]	148 ± 1.4[c]

[a]Weight of gonads × 10^4/weight of animals.
[b]$p < 0.05$.
[c]$p < 0.01$.
[d]$p < 0.001$.
[e]$p < 0.02$.

0.05, and 0.3 mg/kg, it became necessary to conduct research under natural conditions. At the same time the small number of people using these sources did not make it possible to conduct a broad study directed towards investigating the reproductive function of the population. Nevertheless, the questionnaire method was used for studying the sexual function of some men (SFM of the Scientific Research Institute of Psychiatry of the U.S.S.R. Ministry of Public Health).

Analysis of the collected material suggests a tendency towards reduction of function in men who use water with a boron content on the level of 0.3 mg/kg in comparison to the control group of human males (32.24 ± 0.37 for the control group and 27.97 ± 0.49 for the test group, $p < 0.001$).

In summarizing the obtained results, it is necessary to state that the gonadotropic effect served as the primary index in establishing the maximum permissible concentration (MPC) for substances in water reservoirs. The general toxic effect was also taken into consideration in establishing the MPC. In the case of cadmium, the gonadotropic effect was taken into consideration together with the general toxic effect, while in the case of boron, the gonadotropic effect prevailed. This fact emphasizes the urgency of studying the long-term after effects of chemical substances, and we are again reminded that even now it is not enough just to study the toxic properties of chemical compounds with the aim of establishing their hygienic standards. Materials must also be accumulated on the study of these effects for other chemicals.

It should be noted that literature is not unanimous on the action mechanism of cadmium. Some researchers relate the action mechanism to accumulation and to the indirect action of the metal on "critical organs". Others relate this to the effect on the cardiovascular system. A third group of researchers relates this to the effect of cadmium ions on the nuclear substances of the hypothalamus-hypophysis system. The mechanism responsible for the action of boron has been studied even less. Our own studies and the data in the literature make it possible to conclude that boron inhibits the enzymatic regulator of glycosis in the organism. It becomes obvious that the mechanism responsible for the action of cadmium and boron has still not been explained and needs further study.

Thus the cadmium concentration of 0.001 mg/l. should be recommended as the new hygienic standard for cadmium in water reservoirs, while a boron concentration of 0.3 mg/l. is recommended as the hygienic norm for the GOST (state standard) of drinking water.

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